Pathogenicity of Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) Isolates to the Adult Pea Leafminer (Diptera: Agromyzidae) and Prospects of an Autoinoculation Device for Infection in the Field

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ABSTRACT Seventeen isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin and three isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) were evaluated for their pathogenicity to the adult pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), in the laboratory. Flies were contaminated with dry conidia through a velvet material wrapped around the inner side of a cylindrical plastic tube. All the isolates were pathogenic to the pea leafminer, causing mortality between 40 and 100% at 5 d after exposure. The lethal time for 50% mortality (LT_{50}) ranged from 2.6 to 5.4 d, whereas the LT_{90} values varied between 3.2 and 9.1 d depending on the isolate. An autoinoculation device was evaluated in cage field experiments using only one of the virulent isolates, *M. anisopliae* ICIPE 20. The device was loaded with 2–3 g of dry conidia. Mortality of up to 100% was observed in flies captured from fungus-treated cages held under laboratory conditions. The average number of spores picked up by a single fly visiting the device increased with days after inoculation. One day after the inoculation, adults picked up an average of $4.1 \pm 0.7 \times 10^5$ conidia and $39.6 \pm 4.0 \times 10^5$ conidia 5 d after inoculation. Depending on the sampling date, the LT_{50} varied between 1.8 and 3.4 d. Results indicate that some isolates of *B. bassiana* and *M. anisopliae* are highly pathogenic to *L. huidobrensis*, suggesting a potential for their use in the control of this pest. They also suggest the possibility of *L. huidobrensis* suppression with fungi using an autoinoculation device.

KEY WORDS autoinoculation device, *Beauveria bassiana*, leafminer, *Liriomyza huidobrensis*, *Metarhizium anisopliae*

The pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), is a highly polyphagous and invasive pest responsible for major yield losses in flower and vegetable crops (Spencer 1973, Weintraub and Horowitz 1995, Wei et al. 2000, Martin et al. 2005a) and is recorded attacking at least 14 plant families (Spencer 1990). Adults damage crops by puncturing the leaf surface to feed and lay eggs into the tissue. When the eggs hatch, the larvae tunnel within the leaf tissue, forming damaging and disfiguring mines (Spencer 1973, Knodel-Montz et al. 1985, Ameixa et al. 2007). Leaf mines and punctures reduce the photosynthetic ability and the quality of high value horticultural crops (Spencer 1973, 1990; Kox et al. 2005). During outbreaks, severe damage resulting from both adult puncturing and larval-mining can lead to total crop losses (Spencer 1973, 1990). Leafminers are also listed as quarantine pests in the EU Plant Health Directive 2000/29 (EU 2000); hence, in addition to the direct damage, losses also result from the restriction in trade and export markets.

The management of leafminers by both small- and large-scale producers worldwide has commonly relied on the use of chemical insecticides (Murphy and La Salle 1999). However, the indiscriminate and frequent use of these chemicals has resulted in insecticide resistance of flies and elimination of their natural enemies (MacDonald 1991, Weintraub and Horowitz 1995, Murphy and La Salle 1999, Rauf et al. 2000). Other leafminer nonchemical control methods that include using parasitoids (Minkenberg and van Lenteren 1986, Waterhouse and Norris 1987, Johnson 1993), trapping by yellow sticky traps (Price et al. 1981, Bennett 1984), resistant plant varieties (CIP 1993), entomopathogenic nematodes (Walters et al. 2000), and bacteria (Çikman and Çömlekçioìlu 2006, Çikman et al. 2008) have been attempted with varied levels of success.

Entomopathogenic fungi that infect their host through the cuticle offer a better alternative for sapfeeding insects (Poprawski et al. 2000, Inbar and Gerling 2008). Various strains of the hyphomycetous fungi, *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin (Hypo-

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Table 1. Identity of fungal isolates used in the study and their percent germination on SDA at 22–29°C

Fungal species	Isolate	Source	Locality/country	Year of isolation	Percent germination ± SE
Metarhizium anisopliae	ICIPE 18	Soil	Mbita (Kenya)	1989	97.0 ± 1.1abc
	ICIPE 20	Soil	Migori (Kenya)	1989	99.3 ± 0.5a
	ICIPE 40	Soil	Kitui (Kenya)	1990	86.8 ± 2.0e
	ICIPE 41	Soil	Lemba (D.R. Congo)	1990	97.8 ± 0.9abc
	ICIPE 51	Soil	Embu (Kenya)	1989	91.5 ± 1.6cde
	ICIPE 57	Soil	Kericho (Kenya)	2005	92.8 ± 1.1bcde
	ICIPE 60	Soil	Kakello (Kenya)	1990	98.3 ± 1.4ab
	ICIPE 62	Soil	Matete (D.R. Congo)	1990	97.0 ± 1.7abc
	ICIPE 63	Soil	Matete (D.R. Congo)	1990	94.5 ± 1.4abcde
	ICIPE 69	Soil	Matete (D.R. Congo)	1990	98.5 ± 0.7ab
	ICIPE 30	*Busseola fusca*	Kendubay (Kenya)	1989	96.5 ± 1.6abc
Metarhizium anisopliae	ICIPE 78	*Temnoschoita nigroplagiata*	Ungoe (Kenya)	1990	85.8 ± 2.0e
	ICIPE 84	*Ornitacris turbida cavroisi*	Kaffraine (Senegal)	2003	96.0 ± 0.7abcd
	ICIPE 402	Homoptera	Shimba Hills (Kenya)	2007	82.5 ± 2.2e
	ICIPE 315	*Tetranychus urticae*	Kerugoya (Kenya)	2006	96.3 ± 2.2abc
	ICIPE 387	*Forficula senegalensis*	Mai Mahiu (Kenya)	2007	99.0 ± 0.6a
	ICIPE 07	*Rhipicephalus appendiculatus*	Rusinga Island (Kenya)	1996	97.5 ± 0.7abc
Beauveria bassiana	ICIPE 273	Soil	Mbita (Kenya)	2006	97.8 ± 1.3abc
	ICIPE 279	Coleopteran larvae	Kericho (Kenya)	2005	85.3 ± 0.9e
	ICIPE 603	Hymenoptera	Taita (Kenya)	2007	87.8 ± 3.2de

Within columns, means followed by the same letters are not significantly different (Student-Newman-Keuls, $\alpha = 0.05$). $n = 80$.

creales: Clavicipitaceae), have been reported to be virulent to other dipteran pests including house fly (Watson et al. 1995, Renn et al. 1999), tsetse fly (Kaaya and Okech 1990, Maniania 1998), and fruit flies (Dimbi et al. 2003, Quesada-Moraga et al. 2006). There have been few attempts to use entomopathogenic fungi against dipteran leafminers (Bordat et al. 1988, Borisov and Ushchekov 1997), but these studies were limited to the screening of fungal isolates in the laboratory against puparia and not adults. The major challenge for using mitosporic entomopathogenic fungi to control dipteran flies has been their application in the field. Recently, contamination devices were developed to infect fruit flies (Dimbi et al. 2003) and tsetse flies (Maniania 1998, 2002) and were tested in the field with success (Maniania et al. 2006, Ekesi et al. 2007). Therefore, this technique offers new prospects for application of entomopathogenic fungi for the control of dipteran leafminers.

Contamination devices use visual and chemical cues to attract tsetse flies and fruit flies, respectively. The leafminer fly trapping technique using yellow sticky cards has been based on visual cues only, because no known sex pheromone has been identified or isolated. Previous leafminer studies when sticky cards were used have shown an attraction for yellow and green, with yellow being the most common color (Chandler 1981, Zoebisch and Schuster 1990, Martin et al. 2005b). In fact, yellow color trap boards coated with tangle foot and electrically driven backpack suction traps have been used to monitor and reduce leafminer populations (Price et al. 1981, Bennett 1984). The design of a device that will attract leafminers to the foci of inoculum will be of paramount importance in ensuring field fly contamination, thereby enhancing control using fungus.

The objectives of this study were (1) to screen fungal isolates of *Beauveria* and *Metarhizium* for their virulence against the pea leafminer in the laboratory to select the most virulent that could be developed as biocontrol agents and (2) evaluate the performance of an autoinoculation device for infecting adult flies in the field.

Materials and Methods

Insects. Adults of *L. huidobrensis* were obtained from the mass rearing unit at the International Centre of Insect Physiology and Ecology (*icipe*). The leafminer colonies were maintained on faba bean, *Vicia faba* L., in a rearing room (25–27°C, 60–80% RH, with a 12 L:12 D photoperiod). Adults were fed on 10% sugar solution from balls of cotton wool soaked in the solution placed at the bottom corner of the rearing cages. In all the bioassays, 1- to 2-d-old adult flies were used.

Fungal Isolates. All 20 fungal isolates (17 *M. anisopliae* and 3 *B. bassiana*) used in the study were obtained from *icipe* Arthropod Germplasm Centre (Table 1). The isolates were cultured on Sabouraud dextrose agar (SDA) in petri dishes (9 cm) and incubated at 25 ± 2°C in complete darkness. Conidia were harvested from 3-wk-old cultures with a sterile spatula. The viability of conidia was based on germ tube formation (Goettel and Inglis 1997). Conidial suspensions (0.1 ml) titrated to 3×10^6 conidia/ml were spread-plated on petri dishes (9 cm) containing SDA medium. A sterile microscope coverslip (2 by 2 cm) was placed on top of the agar in each plate. Plates were incubated in complete darkness at 25 ± 2°C and examined after 20 h. Percentage germination of conidia was determined by counting the number of germinated conidia (a germ tube two times the diameter of the propagule) from 100 spores counted randomly on the surface area covered by each coverslip under the light microscope (400×) (Goettel and Inglis 1997). Four replicate plates per isolate were used.

Pathogenicity of *M. anisopliae* and *B. bassiana* Isolates Against Adult *L. huidobrensis*. A modified contamination technique described by Dimbi et al. (2003) for fruit flies was used. Adults of *L. huidobrensis* were exposed to 0.1 g of dry conidia evenly spread on a cotton velvet cloth covering the inner side of a cylindrical plastic tube (70 mm length by 48 mm diameter). For each isolate, 25 flies were transferred into the cylindrical tube and allowed to walk on the velvet for 1 min, after which 20 insects were transferred from the velvet into a clean ventilated Perspex cage (150 by 150 by 200 mm). A ball of cotton wool soaked in 10% sugar solution was placed at the bottom corner of the cage as a food source. Insects in the control treatments were exposed to fungus-free velvet cloth before being transferred into similar cages. Treatments were arranged in a complete randomized design and repeated four times. Flies were maintained at 25–27°C, 50–70% RH, under a 12 L:12 D photoperiod.

Mortality was recorded daily for 11 d. Dead insects were surface sterilized in 70% alcohol followed by three rinses in distilled water and transferred to petri dishes lined with damp sterilized filter paper to allow fungal growth on the surface of the cadaver. Dead flies that sporulated on the surface of the cadavers were used for analysis. Five flies that had been left out of the initial 25 were used to estimate the number of conidia picked up by a single fly in each treatment. Insects were transferred into 2-ml cryogenic tubes containing 1 ml of sterile 0.05% Triton X-100. The tube was vortexed for 2–3 min to dislodge conidia from the insect and concentration of conidia was determined using a hemocytometer.

Mass Production of Selected Fungal Isolate. One of the most virulent isolates, *M. anisopliae* ICIPE 20, was selected for field cage experiments to evaluate the performance of an autoinoculation device. Conidia were mass-produced on whole rice substrate in Milner bags (60 cm long by 35 cm wide). Rice was autoclaved for 1 h at 121°C and inoculated with a 3-d-old culture of blastospores (Jenkins et al. 1998). The sterile rice was incubated for 21 d at 20–26°C, 40–70% RH, and allowed to dry for 5 d at room temperature. Conidia were harvested by sifting the substrate through a sieve (295-μm mesh size). The conidia were stored at 4–6°C until used. Viability was determined as described above, and germination of 86–90% was recorded after 20 h.

Description of Autoinoculation Device. After preliminary studies, two types of autoinoculation devices were selected and evaluated for their ability to attract and infect flies. The first prototype was made from 500-ml clear disposable plastic beverage bottles (5.5 cm diameter, 21 cm in height), which will henceforth be called device A. Five entry/exit holes (2 by 3 cm) were made near the top and another set of five near the bottom of the bottle at alternate positions using a pen knife. A dark gray velvet cloth (3.5 diameter by 11 cm height) was used to hold the conidia. It was held in place by gluing it on its smooth side and wrapping it around a smaller plastic diameter bottle (3 cm diameter by 11.5 cm height) exposing the rough surface. To attract the adult leafminers, a yellow netting (3.8 cm diameter by 11 cm height) of 1-mm^2 holes was wrapped around the velvet cloth and tightened with two office pins (Flyingdeer Office Pins No. 2; Zheijiang Flyingdeer Industrial Co., Ltd., Zheijiang, China) to ease cleaning and replenishing of conidia. The smaller plastic diameter bottle was later introduced into the bigger bottle and fixed at the center using a metallic wire, which also served for hanging the device. The second device was a modification of the Lynfield trap (11 cm diameter by 10 cm height; Fig. 1). Holes similar to the ones described above were made on the trap (Fig. 1B1), and velvet (8 cm diameter by 8.5 cm length) and yellow netting (8.9 cm diameter by 8.6 cm length) was wrapped around a smaller inner cylindrical bottle (5.2 cm diameter and 6 cm in height; Fig. 1B2) that was hung in the trap as described above. This device will henceforth be called device B (Fig. 1B3).

Selection of Autoinoculation Device. All faba bean plants used in this study were planted in screen houses (2.8 m length by 1.8 m width by 2.2 m height) in 15-cm pots (five to eight plants per pot), using a mixture of manure and soil in a ratio of 1–5, respectively. After 2 wk, 20 potted plants (≈35 cm in height) were transferred to field cages (2 m height by 2.9 m diameter), where they were arranged in four rows (30-cm interrow and 15-cm intrarow spacing). Tangle foot glue (Tangle-trap; The Tanglefoot Company, Grand Rapids, MI) was smeared around the yellow netting of the autoinoculation devices. These were suspended in the field cage 1 m apart each at canopy level between the two center rows of the bean plants using a string. One hundred 1- to 2-d-old flies were released into the cage. After 8 h, the number of trapped flies was recorded. Treatments were carried out simultaneously in two field cages, and the experiment was replicated four times.

Evaluation of Autoinoculation Device in Field Cage. The performance of device B was assessed in a field cage (2 m height by 2.9 m diameter) between 24 January and 19 February 2009 to determine its efficiency in delivering inoculum into the leafminer population. Four rows of 20 potted 2-wk-old faba bean plants (height of 35 cm) grown as described earlier were placed in a field cage a day before the experiment. Approximately 2–3 g of conidia was spread evenly on the velvet cloth of the autoinoculation device. The yellow netting was wrapped around the velvet cloth containing spores and tightened with two office pins. The inner side of the device and the outer side of the yellow netting were also lightly dusted with conidia using a camel brush to maximize contamination. The device was hung as described earlier and suspended at canopy level (35 cm) at the center between the two middle rows of the bean plants using a string. Five hundred 1- to 2-d-old flies were released into the field cage. A similar trap device without fungus was suspended in another field cage and acted as the control. At 1, 2, 3, 4, and 5 d after exposure, 30 live flies were collected (from each of the two field cages) at random and transferred individually into clean ster-

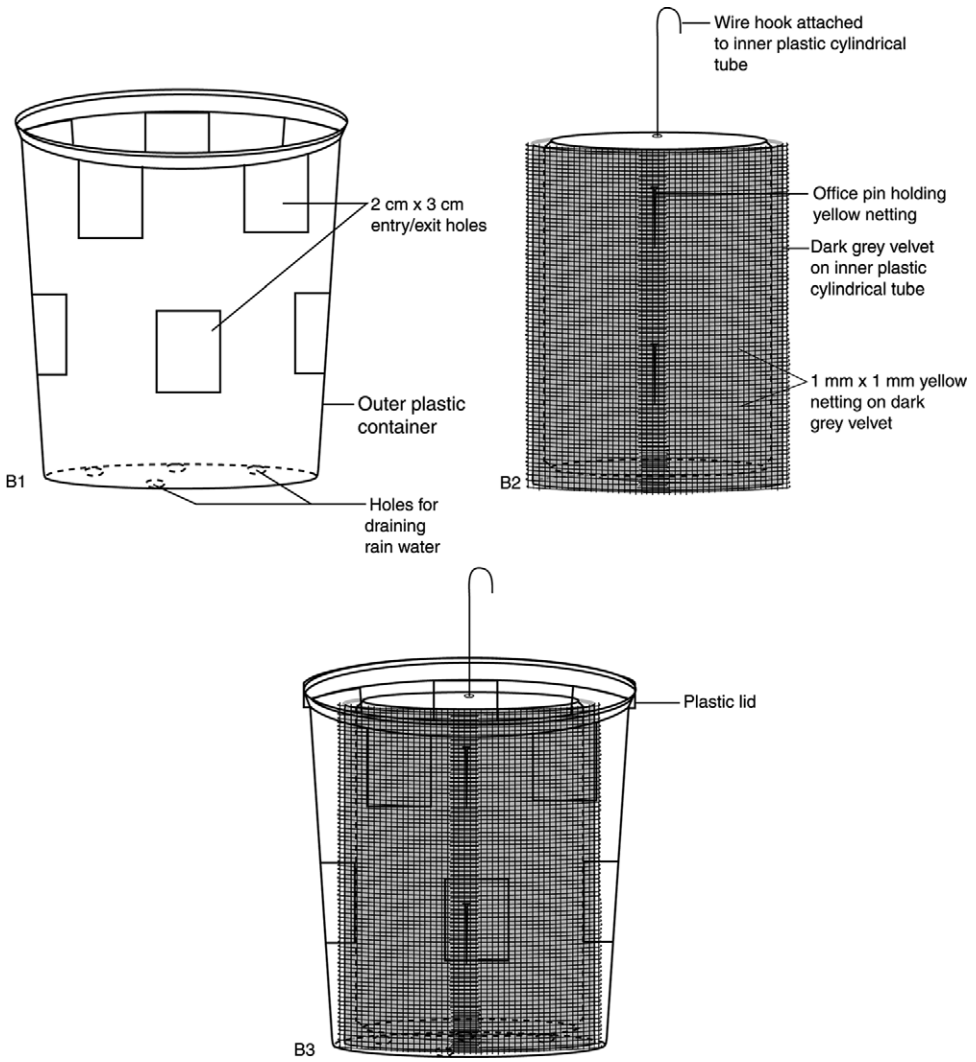

Fig. 1. Autoinoculation device made from modified Lynfield trap (device B).

ile cylindrical plastic tubes (diameter 7 mm by height 49 mm) and brought to the laboratory. Twenty of the 30 tubes containing flies were maintained in an incubator at 25 ± 2°C and 80–90% RH. The mouth of the tube was replaced with a plug of cotton wool soaked in 10% sugar solution as a food source, which was replaced with fresh ones every 2 d. Ten of the remaining tubes with flies were used to estimate the number of conidia picked up by a single fly in each day after exposure using the procedure described earlier. The experiment was replicated four times. Mortality was recorded daily until all the flies died. Dead flies were surface sterilized as described earlier and only mycosed flies were used in the analysis.

The number of pupae produced at the end of the experiment was determined from six potted plants randomly picked from the treatment cages. The potted plants were transferred into rearing cages (0.6 by 0.5 by 0.6 m) until mines were mature. Leaves containing mature mines were harvested and placed on a wire mesh over a tray containing 100 g of sterile sand spread evenly at the bottom of the container to collect pupariating larvae or pupae falling off the leaves. The number of pupae was recorded. The same procedure was used for the control.

Statistical Analysis. Percent mortality was corrected for control mortality (Abbott 1925) and normalized by arcsine transformation (Sokal and Rohlf 1981) before being subjected to analysis of variance (ANOVA) using PROC GLM, at 95% level of significance. Student–Newman–Keuls analysis was used to separate the means as a post-ANOVA procedure ($\alpha = 0.05$). Nontransformed means are presented in the tables. The LT_{50} and LT_{90} values were determined for each replicate using the probit analysis method for correlation data (Throne et al. 1995) and compared among themselves using ANOVA ($\alpha = 0.05$) and means separated using Student–Newman–Keuls. Correla-

Table 2. Virulence of *M. anisopliae* and *B. bassiana* isolates against *L. huidobrensis* adults

Fungal species	Fungal isolate	Percent mortality (mean ± SE)	LT_{50} values (mean ± SE)	LT_{90} values (mean ± SE)	Conidia picked up by a single fly × 10^4 ± SE
Metarhizium anisopliae	ICIPE 18	100a	2.9 ± 0.16hi	3.2 ± 0.1f	12.6 ± 1.4ab
	ICIPE 387	98.6 ± 1.4a	3.3 ± 0.02fg	4.2 ± 0.2ef	8.6 ± 0.6abc
	ICIPE 20	97.4 ± 1.5ab	2.8 ± 0.1hi	3.8 ± 0.2ef	12.5 ± 1.2ab
	ICIPE 84	97.4 ± 1.5ab	2.6 ± 0.04i	3.5 ± 0.1f	15.6 ± 3.0a
	ICIPE 62	96.1 ± 1.3ab	2.8 ± 0.1hi	4.1 ± 0.2ef	9.1 ± 0.6abc
	ICIPE 60	94.6 ± 2.3ab	3.0 ± 0.1gh	3.9 ± 0.6ef	9.0 ± 1.0abc
	ICIPE 07	85.4 ± 2.5ab	3.3 ± 0.1fg	5.3 ± 0.2cd	6.3 ± 0.5cd
	ICIPE 78	82.5 ± 3.7ab	3.4 ± 0.1ef	5.8 ± 0.5c	5.6 ± 0.9cd
	ICIPE 69	78.6 ± 2.5ab	4.0 ± 0.1c	5.9 ± 0.2c	4.7 ± 0.5d
Metarhizium anisopliae	ICIPE 315	76.0 ± 1.8b	3.5 ± 0.1def	6.0 ± 0.4c	4.5 ± 0.7d
	ICIPE 57	57.2 ± 4.9c	4.7 ± 0.1b	7.4 ± 0.3b	4.7 ± 0.6d
	ICIPE 63	55.8 ± 6.2c	4.5 ± 0.1b	7.6 ± 0.3b	4.6 ± 0.5d
	ICIPE 40	51.8 ± 6.9cd	5.2 ± 0.3a	9.1 ± 0.5a	4.4. ± 0.6d
	ICIPE 30	49.1 ± 5.8cd	—	—	4.5 ± 0.4d
	ICIPE 41	47.9 ± 3.2cd	—	—	4.7 ± 0.5d
	ICIPE 402	45.3 ± 2.1cd	—	—	4.4 ± 0.9d
	ICIPE 51	39.9 ± 3.1d	—	—	5.3 ± 0.3cd
Beauveria bassiana	ICIPE 273	100a	2.9 ± 0.1hi	3.8 ± 0.2f	7.7 ± 0.6bcd
	ICIPE 603	93.4 ± 1.3ab	3.7 ± 0.1cde	4.6 ± 0.1de	6.1 ± 1.2cd
	ICIPE 279	93.3 ± 2.6ab	3.8 ± 0.1cd	4.7 ± 0.3de	8.0 ± 1.1bcd
	Control	6.3 ± 1.3e	—	—	—
		n = 84	n = 64	n = 64	n = 100

Within columns, means followed by the same letters are not significantly different, ANOVA and Student-Newman-Keuls comparisons of means, $\alpha = 0.05$.

Percent mortality, lethal time mortality (LT_{50} and LT_{90}) (mean ± SE) 5 d after inoculation and the mean no. of conidia per fly in the laboratory.

tion analysis was carried out to relate mortality rate with mean number of conidia picked up by a single fly from the contamination tube. Repeated-measures analysis (PROC GLM-Wilks' Lambda statistic) was used to relate the number of spores picked by a single fly from the autoinoculation device to the different sampling days. Paired *t*-test was used to compare the performance of the two devices. The number of pupae produced in the treatments and those in the control were analyzed using the Wilcoxon-Mann–Whitney *U* test. All analyses were performed using the SAS (SAS Institute 2003) version 9.1 statistic package.

Results

Pathogenicity of *M. anisopliae and B. bassiana* Against the Adult *L. huidobrensis*. Germination of conidia of different fungal isolates used in the study ranged from 83 to 99% (Table 1). The number of conidia picked up by a single fly from the contamination tube was significantly different among the isolates (F = 9.02; df = 19,80; $P < 0.0001$) and ranged between 4.4×10^4 and 15.6×10^4 conidia per fly (Table 2). There was a positive correlation between mortality rates and the amount of conidia picked by a single fly for the different isolates (r = 0.67; N = 84; $P < 0.0001$). The mean mortality in the controls was 6.3 ± 1.3% 5 d after inoculation. Both species of fungi were pathogenic to adult *L. huidobrensis;* but mortality varied significantly among the isolates (F = 54.17; df = 20,63; $P < 0.0001$) 5 d after infection (Table 2). Mortality ranged between 39.9% for the least virulent isolate (*M. anisopliae* ICIPE 51) and 100% for the most virulent isolates (*M. anisopliae* ICIPE 18 and *B. bassiana* ICIPE 273). Mortalities of adult *L. huidobrensis* inoculated with *B. bassiana* species did not differ significantly, and the average ranged between 93 and 100%. The lethal time was calculated for 16 isolates that caused >50% mortality. The LT_{50} values were significantly different among the 1isolates (F = 64.64; df = 15,48; $P < 0.0001$) and ranged between 2.6 and 5.2 d. Similarly, the LT_{90} values were significantly different among the isolates (F = 33.49; df = 15,48; $P <$ 0.0001) and ranged between 3.2 and 9.1 d (Table 2).

Selection of Autoinoculation Device. Device B made from the modified Lynfield trap attracted more flies (49.0 ± 11.7) than did the device A (15.9 ± 4.7; t = 8.23; $P < 0.0001$) and was subsequently selected for further studies.

Evaluation of Autoinoculation Device in Field Cage. The mean number of spores picked up by a single fly visiting device B increased steadily (F = 56.78; df = 4,36; $P < 0.0001$) with days after inoculation. One day after the inoculation, individual adult flies picked up an average of 4.1×10^5 conidia in contrast to 39.6×10^5 conidia 5 d after inoculation (Table 3). Mortality of the flies in the control cages ranged between 0 and 35% (Table 3). Mortality of flies collected from the fungus-treated cage on 2–5 sampling d was significantly different from 1-d sampling (F = 19.46; df = 4,15; $P < 0.0001$; Table 3). The LT_{50} values varied among the sampling dates (F = 8.85; df = 4,15; $P < 0.0007$), the shortest (1.8 d) being at 3 d after exposure and the longest (3.4 d) at 1 d after exposure (Table 3). The cumulative mortality in the different sampling days increased progressively, reaching 100% over 6 d after treatment (Fig. 2). There was no significant difference between the number of pupae col-

Table 3. Mean percentage mortality (mean ± SE) of control and fungal infected adult *L. huidobrensis* at 4 d after exposure, LT_{50} values, and mean no. of conidia per single fly exposed to *M. anisopliae* isolate ICIPE 20

Sampling days after inoculation	Percent mortality of control (mean ± SE)	Percent mortality of infected (mean ± SE)	LT_{50} values (mean ± SE)	Mean no. conidia/fly × 10^5 (mean ± SE)
1	0b	68.4 ± 4.9b	3.4 ± 0.1a	4.1 ± 0.7d
2	28.75 ± 2.39ab	100a	2.4 ± 0.2bc	12.3 ± 1.5c
3	30.00 ± 2.89ab	100a	1.8 ± 0.2c	25.1 ± 2.1b
4	25.00 ± 2.89ab	97.8 ± 2.2a	2.3 ± 0.2bc	32.3 ± 3.3b
5	35.00 ± 2.04a	98.2 ± 1.8a	2.6 ± 0.2b	39.6 ± 4.0a

Means within columns followed by same letter are not significantly different, ANOVA and Student-Newman-Keuls comparisons of means, $\alpha = 0.05$. $n = 20$.

lected in the control (2976.0) and fungus treatments (2,400.8; $Z = -1.9$; $P < 0.1$).

Discussion

The potential of entomopathogenic fungi for control of insect pests has been shown (Zimmermann 1986, Ferron et al. 1991, Inglis et al. 2001). However, the success in the development of entomopathogenic fungi as mycoinsecticides involves several steps including, isolation from the environment or diseased insects, strain selection based on several selection criteria such as virulence, and pathogen storage properties and formulation (Soper and Ward 1981, Butt and Goettel 2000). The results of laboratory screening have shown that all 20 fungal isolates were pathogenic to adult *L. huidobrensis.* There was, however, considerable variation in the virulence among fungal isolates as shown by mortality and lethal time values. The intraspecific variations in the pathogenic activity of entomopathogenic fungi observed in our study is similar to those reported for other arthropod pests (Ekesi et al. 1998, Dimbi et al. 2003, Quesada-Moraga et al. 2006, Bugeme et al. 2009), including leafminer immature stages (Bordat et al. 1988, Borisov and Ushchekov 1997). In our study, none of the tested isolates originated from the host or closely related species, showing that isolates of diverse origin can be equally pathogenic to *L. huidobrensis.* These results also show that some isolates of both *M. anisopliae* and *B. bassiana* are highly virulent to *L. huidobrensis.*

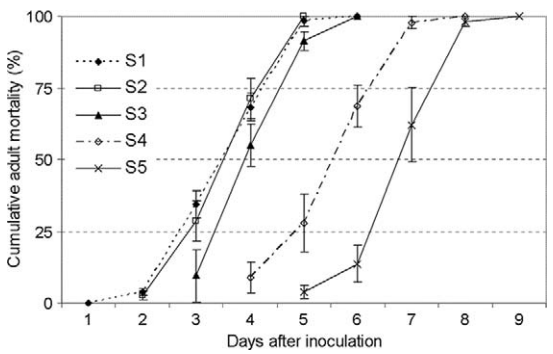

Fig. 2. Infectivity of *M. anisopliae* applied in inoculation device showing cumulative mortality of adult *L. huidobrensis* over 6 d collected after different sampling days (S1–S5). $n = 80$.

The number of conidia picked up by a single fly from the contamination tube ranged between 4.4×10^4 and 1.6×10^5. Using a similar infection technique, Dimbi et al. (2003) obtained between 4.2×10^5 and 1.0×10^6 conidia per single fruit fly. This difference could be attributed to the amount of conidia (0.1 g) used in our study compared with 0.3 g of conidia used by Dimbi et al. (2003), and the size of fruit flies, which are bigger than leafminer flies. The percent mortality was positively correlated with the amount of conidia picked by a single fly from the contamination tube, suggesting that fungal infection of leafminers will depend on, among other factors, the pathogen properties such as ability to adhere to the host cuticle and the type of formulation.

Autodissemination devices have been developed, whereby insects are used to vector inoculum among conspecifics in the environment after they have been attracted and acquired the pathogen (Vega et al. 2007). This technique has been successfully tested against tsetse flies (Maniania et al. 2006) and fruit flies (Ekesi et al. 2007). Subsequently, for this study, an autoinoculation device was developed from a modified Lynfield trap and tested in field cage experiments. Based on this study, leafminer flies were attracted to the device and were able to pick up a lethal dose of inoculum (4.1×10^5–4.0×10^6 conidia per fly), resulting in higher adult mortality. Flies exhibited different behaviors inside the traps: (1) some flies landed on the device and almost exited immediately; (2) others walked on the sides of the device before exiting; (3) some entered the trap and walked on the inner side of the trap before exiting; and (4) others were seen jumping up and down and either wriggled or groomed before exiting. This corroborates with the observations by Maniania (2002), who reported that insect behavior could affect the amount of inoculum picked by a single fly in the contamination device.

The performance of the selected autoinoculation device was studied in terms of delivering conidia into fly populations in field cages. All flies sampled at different time intervals (1–5 d after introduction of the treated device) succumbed to fungal infection with development of mycosis caused by *M. anisopliae*, which is an indication of successful contamination of flies through the device. However, our results still do not address the issue of fly-to-fly transmission, which is fundamental to the success of this technique. Further studies will be needed to show whether a proportion of fungus-infected flies could transfer infection to the healthy ones. The

viability of spores in the device over time was also not studied. However, Maniania (1998) reported that the pathogenic activity of *M. anisopliae* conidia to tsetse flies remained unchanged at 8 d after exposure and could retain their infectivity for >21 d when applied to an infection chamber under field conditions. The device made is cost-effective because materials used are locally available, and the device can be produced locally. Once produced, the device can be reused over a long period of time requiring only cleaning and replenishing of conidia.

The number of pupae collected from the control and fungus treatments were not significantly different in this study. There is no clear explanation for these results but the fact that not all flies became infected at the same time and that adults do not die immediately after infection implies that flies still have time to lay eggs before dying. These results emphasize the need for timing fungal application before population build-up if full use of the pathogen is to be made. It further emphasizes the need for integration of entomopathogenic fungi with other pest control strategies for the management of leafminers.

The results of our study suggest that *M. anisopliae* (ICIPE 315, 69,78, 07, 60, 62, 84, 20, 387, and 18) and *B. bassiana* (ICIPE 273, 603, and 279) could be considered for further development as microbial control agents of the pea leafminer, and the contamination device could further be evaluated for field application of fungus for adult leafminer management.

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